LESSON 10: ORGANOGENESIS

Objective

To understand the pattern of organ development in cultures, you should first understand the basic concept of totipotency. Two concepts, plasticity and totipotency are central to understanding cell culture and regeneration. Plants, due to their sessile nature and long life span, have developed a greater ability to endure extreme conditions and predation than have animals.

Many of the processes involved in plant growth and development adapt to environmental conditions. This plasticity allows plants to alter their metabolism, growth and development to best suit their environment. Particularly important aspects of this adaptation, as far as plant tissue culture and regeneration are concerned, are the abilities to initiate cell division from almost any tissue of the plant and to regenerate lost organs or undergo different developmental ways in response to particular stimuli.

What is Totipotency?

When plant cells and tissues are cultured in vitro they generally exhibit a very high degree of plasticity, which allows one type of tissue or organ to be initiated from another type. In this way, whole plants can be subsequently regenerated. This regeneration of whole organisms depends upon the concept that all plant cells can, given the correct stimuli, express the total genetic potential of the parent plant. This maintenance of genetic potential is called 'totipotency'. Plant cell culture and regeneration do, in fact, provide the most compelling evidence for totipotency. In practical terms though, identifying the culture conditions and stimuli required to manifest this totipotency can be extremely difficult and it is still a largely empirical process.

Organogenic differentiation is an outcome of the process of dedifferentiation followed by redifferentiation of cells. The terms dedifferentiation and redifferentiation have already been described in Lesson 1 (Unit 1). Dedifferentiation favours unorganized cell growth and the resultant developed callus has meristems randomly divided. Most of these meristems, if provided appropriate in vitro conditions, would redifferentiate shoot buds and roots. Mostly, the whole plant regeneration from cultured cells may either occur through shoot-bud differentiation or somatic embryogenesis. This establishes the totipotency of somatic cells which, under natural conditions, can also be observed to some extent during the vegetative reproduction of plant species. The cell(s) of stem, leaf and root cuttings of several plants are able to directly differentiate shoots and roots.

The earliest reports on controlled organogenesis in vitro were by White 1939 who obtained shoots on callus of a tobacco hybrid and by Nobecourt 1939, who observed root formation in carrot callus. The finding of White was confirmed and extended by Skoog 1944, who showed that auxin could stimulate rooting and inhibit shoot formation. Further studies established that a balanced cmbination of auxin and cytokinin controls the root and shoot formation (Skoog and Miller, 1957).

Organogenesis in Tobacco (Nicotiana tabacum)

Organogenesis from tobacco pith callus is the classical example of how varying plant growth regulator regimes can be used to manipulate the pattern of regeneration from plant tissue cultures. When cultured on a medium containing both auxin and cytokinin, callus will proliferate. If the auxin to cytokinin ratio is increased, adventitious roots will form from the callus by organogenesis. It the auxin to cytokinin ratio is decreased adventitious shoots will be formed. If the explants are cultured on medium containing only a cytokinin shoots can be produced directly.

Tobacco plants can also be easily regenerated from tobacco leaf pieces. Leaves are cut into aproximately 1 cm squares with a sterile scalpel (avoiding large leaf veins and any damaged areas). The leaf pieces are then transferred (right side up) to gelled MS medium supplemented with 1mg /l BAP (a cytokinin) and 0.1mgl/1 NAA (an auxin). Over the next few weeks, callus forms on the explants, particularly around the cut surfaces. After 3 to 5 weeks shoots emerge directly from the explants or from callus derived from the explants. When these shoots are about 1cm long they can be cut at the base and placed on to solid MS medium without any plant growth regulators. The shoots will form roots and form plantlets that will grow in this medium and can subsequently be transferred to soil.

So, the auxin to cytokinin ratio of the medium determines which developmental pathway the regenerating tissue will take. It is usual to induce shoot formation by increasing the cytokinin to auxin ratio of the culture medium. These shoots can then be rooted relatively simply.

Factors affecting Regeneration

Regenaration via de novo organogenesis is a complex multi-staged phenomenon. There are many factors which influence the process. In general, organized development is successfully achieved through proper selection of the inoculum, proper choice of the medium, a balanced combination of plant growth regulators and the control of physical environment. There is no common formulation for all or many materials. The major factors affecting the process of regeneration are presented here:

Source of Explant

The factors which influence the response of the inoculum in the culture are:

1. The organ that is to be served as tissue source
2. The physiological and ontogenic age of the organ
3. The season in which the explant is obtained
4. The size of the explant

5. The overall quality of the plant from which explants are taken.

Regenaration from any plant part can be achieved by proper combination of the factors. Stem segments and apices, root segments, leaf pieces, petioles, inflorescence sections, flower petals, ovular tissue, seedling parts (cotyledon, hypocotyl) and seed embryos have been used as an explant. All these explants can give rise to organs and embryos directly or indirectly via callus. As mentioned above a suitable explant is desirable for a given species for successful regeneration, e.g., embryonic tissues as an explant for regenaration in cereals. Explants consisting of actively dividing cells have been useful in initiating the cultures and subsequent regeneration.

Nutrient Media and Constituents

Following medium constituents influence the regeneration more profoundly

1. Inorganic macro- and micro-nutrients – mostly MS and B5 media used
2. Carbon (energy) source – generally, 2-4% sucrose used
3. Reduced nitrogen source
4. Plant growth regulators – auxins (2,4-D, IAA, NAA and IBA) and cytokinins (kinetin and BA)
5. Vitamins – Inositol, Nicotinic acid, Pyridoxine and Thiamine.

Culture Environment

These include:

1. physical form of the medium i.e. presence or absence of agar
2. the pH of the medium
3. light quality and quantity
4. temperature
5. relative humidity, and
6. the gaseous atmosphere within the vessel.

Regulation of Regenaration

Using a suitable explant and a proper medium callus or explants can be induced to form organs . It is species specific. Plant growth regulators play the most important role in these processes.

Many growth active substances, plant growth regulators as well as other nutrients, have been included in the culture medium to regulate organogenesis in vitro.

As stated earlier, most of the plant species respond to a suitable auxin/ cytokinin balance by forming shoots and roots. Evans and coworkers (1981) found that for 75% of the species forming shoots, either kinetin or BA was used in a concentration range of 0.05- 46uM. Auxins such as IAA and NAA are used in concentrations of 0.06- 27uM. Graminaceous species e.g alfalfa callus tend to have a lower requirement for cytokinin for shoot formation than other species. Instead, it requires high level of auxin (2,4-D) for shoot formation. IBA and NAA are the most commonly used auxins for rooting. 2,4-D is seldom used in regenaration studies, since it promotes cell proliferation and suppresses cellular and organ differentiation in dicots. A variety of substituted purines, pyrimidines and ureas have been used as cytokinins to achieve organogenesis. Sometimes Gibberellins and Abscisic acid are also used in the medium. Other compounds affecting organogenesis are: amino acids, antiauxins, polyamines, oligosaccharides, etc.

Mechanism of Organ Development

The condition to achieve organogenesis, optimal conditions of medium constituents is determined emperically. This is essential for each species or even each cultivar specifically for clonal propagation. It is apparent that the process of differentiation begins with the changes in individual cells in response to external stimuli. The cells determined to divide and produce callus are changed to produce an organized structure by cellular differentiation.

The morphogenetic signals set up the conditions, which allow competent cells to undergo their internally controlled programme of differentiation. Such a programme results from selective gene action with the subsequent cellular processes of DNA replication and translation. This would result in biochemical and biophysical changes in the target cells, including a change in the metabolic activity. This is followed by cytological and histological changes leading to visible appearence of organs formation.

Shoot regeneration is markedly affected by the genotype of explant in that different varieties of a given species show quite different frequencies of shoot regeneration. In alfalfa, breeding and selection drastically increased regeneration ability. In wheat, callus growth and regeneration ability are governed by genes called, tissue culture response (TCR) genes, which have been mapped on specific chromosomes.

There are sufficient evidences that the process of differentiation begins at single cell level but this does not mean that all organs are produced from single cell. Once the stimulus is set in, centers of meristematic activity are formed surrounding the cell from which the process starts. The organs are developed from these multicellular meristematic centers. Clusters of meristematic cells , called nodules or meristemoids arise in areas that accumulate starch, which is believed to serve as an energy source for shoot bud differentiation. Meristemoids may develop vascular elements inside them, while their outside may be made of cambium like cells. Initially, the meristemoids may either produce a root or shoot. In, general, roots originate from inside the meristemoids (endogenous origin), while shoots develop from outside (exogenous origin), but in some cases shoots originate endogenously.

Conclusion

Organogenic differentiation is an outcome of the process of dedifferentiation followed by redifferentiation of cells. Dedifferentiation favours unorganized cell growth and the resultant developed callus has meristems randomly divided. Most of these meristems, if provided appropriate in vitro conditions, would redifferentiate shoot buds and roots (whole plant regeneration). This establishes the totipotency of somatic cells to undergo regeneration. This Lesson, thus gives you a brief idea as to how organs differentiate from single cells or callus in vitro.

Questions

1. Define Totipotency and discuss its role in organogenesis.
2. What are the factors responsible for regeneration of plants?
3. Giving one classical example of organogenesis, discuss the role of plant growth regulators affecting the process.
4. Explain the basic mechnism of organ development in tissue culture plants.

Note